Kinetics of Huperzine A Dissociation from Acetylcholinesterase via Multiple Unbinding Pathways

Supporting Information

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S1. MOLECULAR DYNAMICS SIMULATIONS

TABLE S1. Summary of the MD simulations performed in this study.

type	RP	time [ns]	information
unbiased MD	—	5	equilibration
biased MD	—	20	reaction pathways
metadynamics	pwf	1400	free energies
metadynamics	pws	1000	free energies
unbiased MD	pwf	800	kinetic rates
unbiased MD	pws	800	kinetic rates

S2. REACTION PATHWAYS

TABLE S2. List of the *Tc*AChE residues, which pair with hupA by hydrogen bonds, occurring along the dissociation pathways pwf and pws and the corresponding center-of-mass distance of these residues to the initial conformation of hupA.

pathway	residue	distance [Å]
pwf and pwf	Pro86	13.77
	Arg88	16.87
	Trp84	5.29
	Asn66	14.35
	Val71	9.32
	Gly80	8.88
	Phe75	10.94
	Glu73	11.74
	Asn87	13.80
	Tyr70	8.75
	Asn85	7.45
	Met90	15.17
	Gly123	8.65
	Ser124	10.54
	Ser122	6.87
	Tyr334	8.00
	Glu278	13.98
	Gln69	10.22
	Tyr121	7.15
	Gln74	10.87
	Ser81	7.06
	Gly77	14.16
	Asp72	6.72
	Pro86	10.35

S3. RATE ESTIMATION

The transition matrix $\mathbf{N}$ was built by counting the number of observed transitions from configuration i to j in the collective variable space within a specified lag time. The transition matrix contains all information needed to estimate the rate matrix $\mathbf{T}$

$$T_{i\ i+1} = \frac{1}{\Delta t} \frac{N_{i\ i\pm1} + N_{i\pm1\ i}}{N_{i\ i}\, \mathrm{e}^{-\beta(F(s_{i\pm1})-F(s_i))} + N_{i\pm1\ i\pm1}}, \tag{1}$$

and

$$T_{i\ i} = -T_{i\ i+1} - T_{i\ i-1}, \tag{2}$$

which were calculated to provide an initial guess of $\mathbf{T}$. Next, a Monte Carlo sampling was used to maximize the log-likelihood l and, in consequence, $\mathbf{T}$. At every iteration, a random element of $\mathbf{T}$ was perturbed by adding a random number ϵ from $(-0.05/\Delta t, 0.05/\Delta t)$. This required a modification of the neighboring elements of $\mathbf{T}$

$$T_{j\ i} \equiv T_{j\ i} + \epsilon\, \mathrm{e}^{-\beta(F(s_j)-F(s_i))}, \tag{3}$$

$$T_{i\ j} \equiv T_{i\ j} - \epsilon\, \mathrm{e}^{-\beta(F(s_j)-F(s_i))} \tag{4}$$

and

$$T_{i\ i} \equiv T_{i\ i} - \epsilon. \tag{5}$$

These modifications were accepted with the Boltzmann probability $\min(1, \mathrm{e}^{\Delta l})$, in which Δl is the difference between the old and new log-likelihood.

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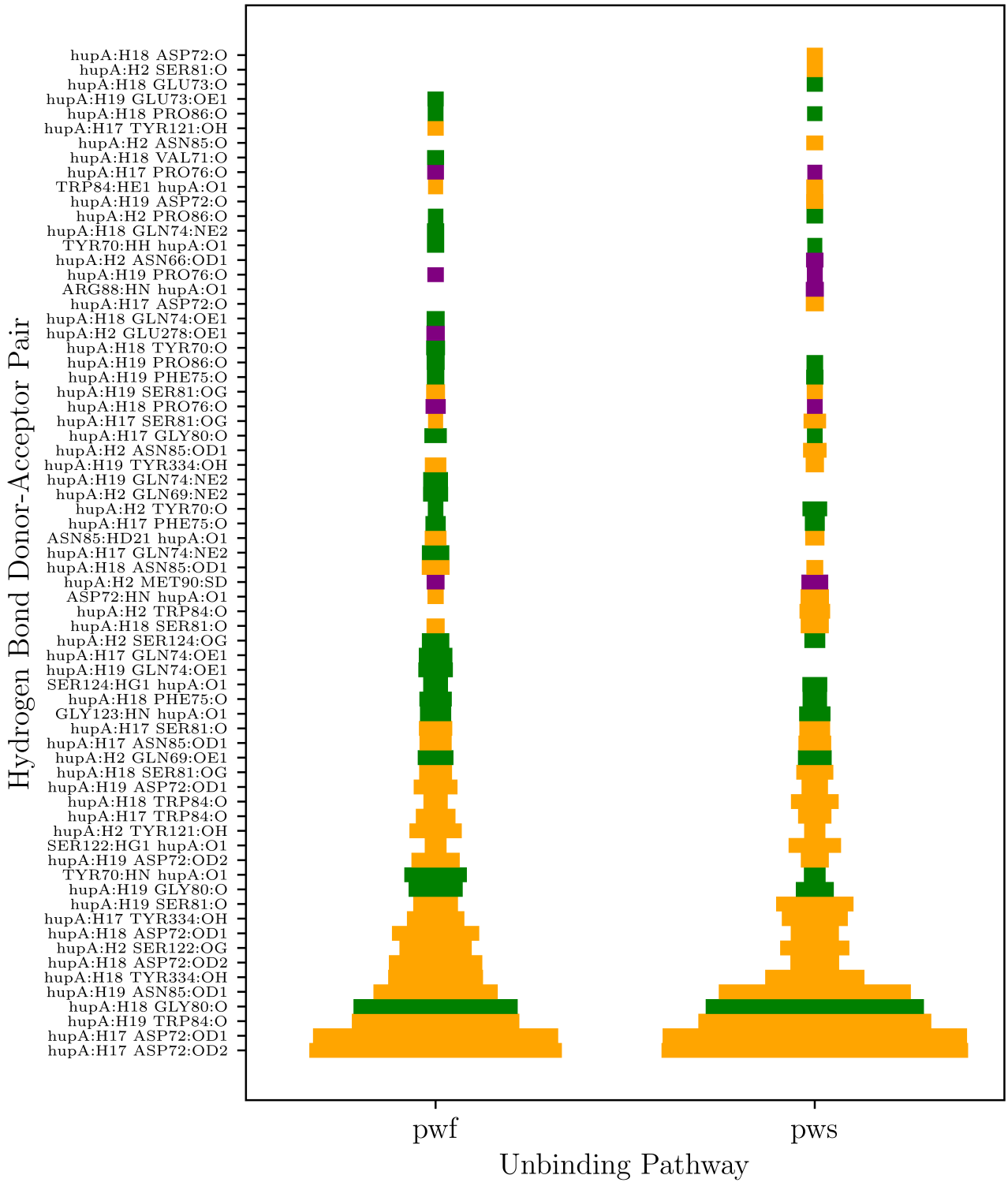

FIG. S1. Interaction frequency for hydrogen donor-acceptor pairs from the MS simulations. Horizontal bars are for individual hydrogen bond donor-acceptor pairs, which are indexed by the vertical axis and sorted by total frequency from the bottom to the top. Graphs are shown for each unbinding pathway as labeled on the horizontal axis. The total width of the bar corresponds to the total frequency of the interaction for the unbinding pathway. A hydrogen bond was counted between a donor-acceptor pair if the distance between the atoms was less than 4.1 Å and the donor-hydrogen-acceptor angle was within 100 and 180 degrees. The residues are clustered by the euclidean distances (Tab. S2) to the initial configuration of hupA using the Jenks optimization algorithm into 3 clusters with a GVF value (goodness of variance fit) of 0.88. The residues are colored by their cluster number: bound–orange, transition–green and unbound–purple.

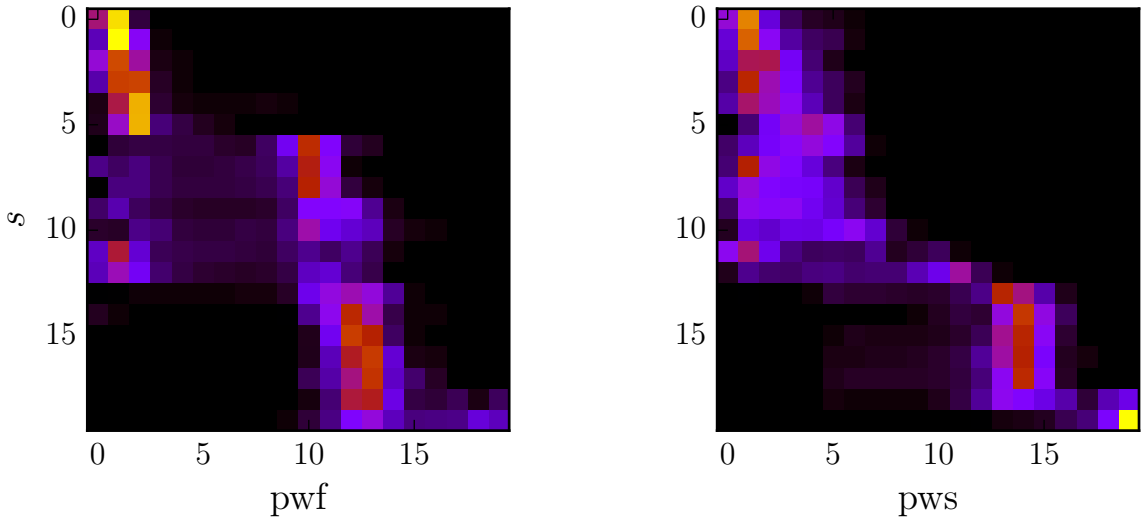

FIG. S2. Transition matrices $\mathbf{N}$ for $\Delta t = 2$ ps calculated using the unbiased MD simulations for pwf and pws. The unbiased trajectories were run from each configuration of the hupA-inhibited *Tc*AChE, starting from $s = 1$ and ending at $s = 20$. For each configuration along the RPs, 20 unbiased MD simulations were performed, resulting in $2 \times 20 \times 20 \times 2$ ns $= 1.6$ μs of the simulation time.

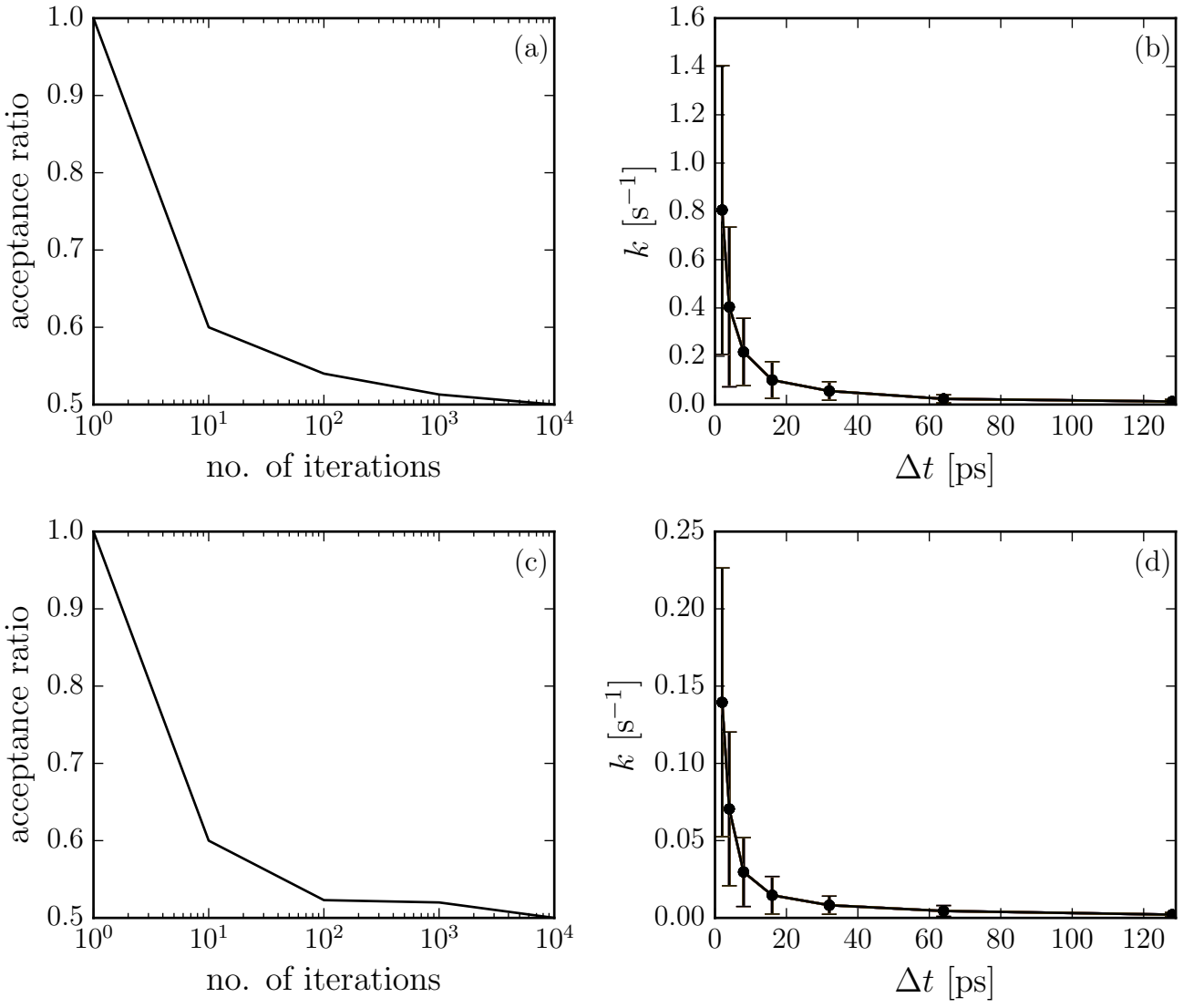

FIG. S3. Metropolis-Hastings acceptance ratios of the algorithm to estimate the s-dependent diffusion coefficients converge to 0.5 within 10000 iterations. The results are shown in (a) and (b) for pwf and (c) and (d) for pws. The average kinetic rates estimated for different values of the lag time Δt from 100 Monte Carlo simulations (a maximum-likelihood approach) for each lag time. The results show similar kinetic rates and decreased errors for $\Delta t > 64$ ps.